

**SPERMATIC EFFECTS OF SHORT-TERM ADMINISTRATION OF AQUEOUS *COLA ACUMINATA* SEED EXTRACT IN WISTAR ALBINO RATS**

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**ABSTRACT**

*Cola acuminata* (kola nut) is an important medicinal plant which is widely consumed. It is claimed locally by some to cause low sperm count. The present study was undertaken to investigate the effect of thirty days oral treatment with aqueous *C. acuminata* seed extract on sperm indices. The study was carried out at two different dose levels of 100 and 200 mg/kg body weight using Wistar albino rats. Control group received distilled water. Epididymal sperm was collected and analyzed using standard methods. Phytochemical analysis of extract showed very high amount of alkaloids and high amounts of saponins and tannins. Extract caused significant ( $p < 0.0001$ ) decrease in sperm count. Extract caused marked reduction ( $p < 0.0001$ ) of proportion of actively motile sperms and simultaneously increased ( $p < 0.0001$ ) immotile sperm proportion, but slow motile sperms were not affected. Furthermore, proportion of sperms with normal morphology was decreased ( $p < 0.0001$ ) by extract while the reverse effect was seen on abnormal sperm morphology. There was no change in sperm viability. The result demonstrates that *C. acuminata* seed does not affect sperm viability, but decreases sperm count, motility and normal morphology in male rats.

**KEYWORDS:** *Cola acuminata*, infertility, kola nut, spermatogenesis, sperm motility, testis.

**INTRODUCTION**

Normal gonadal function is essential for fertility. In the male, the gonads (or testes) produce spermatozoa which fertilize the egg in the female for conception. Problems with gonadal function results in infertility which has been a serious challenge to humans. Infertility among couples is one of the most prevalent health challenge in Sub-Saharan Africa.<sup>[1,2]</sup> The testis, which is the primary male reproductive organ, is very sensitive to xenobiotics and several agents including drugs and medicinal plants have been implicated in its dysfunction.<sup>[3,5]</sup> Some medicinal plants have also been identified to improve testicular activity or helpful in treatment of sexual dysfunction as aphrodisiacs.<sup>[6]</sup> Unfortunately, the potential reproductive influence of some medicinal plants that are frequently consumed such as *Cola acuminata* are not yet well documented.

*Cola acuminata* (Family, Sterculiaceae) is an important medicinal plant with wide applications. It is indigenous to West Africa, especially the countries of Sierra Leone, Liberia, Ivory Coast and Nigeria, but has been distributed to Gabon and Congo. The seed of *C. acuminata* is commonly known as “kola nut” and contains caffeine as one of the major active ingredients. The nut is popularly used as a stimulant and it increases

alertness, wakefulness and energy.<sup>[7,8]</sup> It is also used as a masticatory but chronic use of kola nut results in discoloration of the teeth, which is common among chronic users. Other uses include treating conditions like cough, diarrhea, fever and dysentery.<sup>[9,10]</sup> Some of these medical benefits have been attributed to potent multi-phytochemicals that have been found to be present in kola nut like alkaloids, saponins, tannins, cardenolides and phenolic compounds.<sup>[10,11]</sup> In addition, many African cultures use kola nut during ceremonial activities as symbol of peace, friendship and hospitality.<sup>[12,13]</sup> Some also rely on kola nut for their religious activities like incarnations and divinations.<sup>[13]</sup>

Furthermore, the plant is believed locally to have aphrodisiac property, but this is yet to be established. Also; it is believed by some that it induces low sperm count, but there is no evidence to this claim. Hence, the influence of the plant on male reproduction becomes a concern. It is in the light of the above that this study was undertaken to evaluate the effect of sub-acute oral administration of aqueous extract of *Cola acuminata* seed on sperm indices- sperm count, motility, viability and morphology in the Wistar rat.

## MATERIALS AND METHODS

### Plant sample preparation and phytochemical screening

*Cola acuminata* seeds were obtained from a fruit and vegetable market (Ikwerre Road, Port Harcourt, Nigeria). The plant was authenticated by Dr. Chimezie Ekeke of Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria and assigned a reference number (UPH/C/079) which was deposited at the University's herbarium. The seed was reduced in size initially and pulverized with a blender after air-drying. The plant material was then extracted by cold maceration (1:5) with distilled water using for 24 h at room temperature with continuous agitation. Extract was filtered and concentrated under reduced pressure using a rotary evaporator and a reddish-brown powder was obtained with a yield of 5.1%. The extract was stored in an amber colored bottle and preserved in a refrigerator until used for experiments.

Phytochemical screening of the extract was done for presence of alkaloids, saponins, tannins, flavonoids, steroids, anthraquinones, cardenolides and carbohydrates using standard procedures.<sup>[14]</sup>

### Experimental design

Fifteen male Wistar albino rats of body weight 180-200 g were used for the study. They were obtained from the Animal House of the Department of Experimental Pharmacology and Toxicology, University of Port Harcourt, Nigeria and maintained under standard laboratory conditions at room temperature in 12 h photoperiod. They were fed with rat feeds and tap water was given *ad libitum*. The animals were acclimatized for 2 weeks before commencement of the experiment. All experiments were conducted in compliance with standard guidelines for care and use of laboratory animals.

The rats were divided into two experimental groups (containing 5 each) and administered with 100 or 200 mg/kg *Cola acuminata* extract by oral gavage daily for 30 days. A third group was administered distilled water and served as the control. The doses used were based on previous similar studies,<sup>[15,16]</sup> and lower than the oral lethal dose (LD<sub>50</sub>) of the plant, 5000 mg/kg.<sup>[17]</sup>

The animals were deeply anaesthetized with diethyl ether at the end of extract administration and sacrificed by cervical dislocation. The testis was removed and sperm was collected from the epididymis and sperm motility, count, viability and morphology were estimated using standard procedures.<sup>[18]</sup> Briefly, undiluted sperm was placed on a glass slide and covered with a cover slip. This was examined under a light microscope (Surgifield Medicals, England) using 400x objective to assess sperm motility. Sperm motility was graded as: rapid progressive motility, slow or sluggish progressive motility, and immotility. Sperm count was determined using the hemocytometer. Diluted sperm sample (1:20) was placed in a hemocytometer and allowed to stand in a moist

chamber for 15 mins and complete morphologically mature sperm cells were counted using 400x magnification. For sperm viability, a drop of sperm was placed on a slide and one drop of 0.5% eosin stain was applied and left for about 2 min at room temperature. A smear was made and slide was examined under the microscope. About one hundred spermatozoa were viewed at random and the cells that were colored orange-red were believed to have allowed stain to pass through their membranes and considered dead, while the cells that had no stain were considered alive. For sperm morphology, sperm smears were stained on microscopic slides with two drops of Walls and Ewas after air-drying. Morphological characteristics were examined under brightfield optics at 1000x magnification with oil immersion. About one hundred spermatozoa were counted and the percentage of abnormal forms (morphology) was calculated.

### Statistical analysis

Data obtained were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple posttest using GraphPad Prism 5.0 software. P values < 0.05 were considered significant.

## RESULTS

### Phytochemical screening

Phytochemical screening of extract showed the presence of alkaloids, flavonoids, tannins, cardenolides, anthraquinones and saponins (Table 1). Alkaloids were most abundant, while tannins and saponins were moderately present. The other phytochemicals were sparsely present (Table 1)

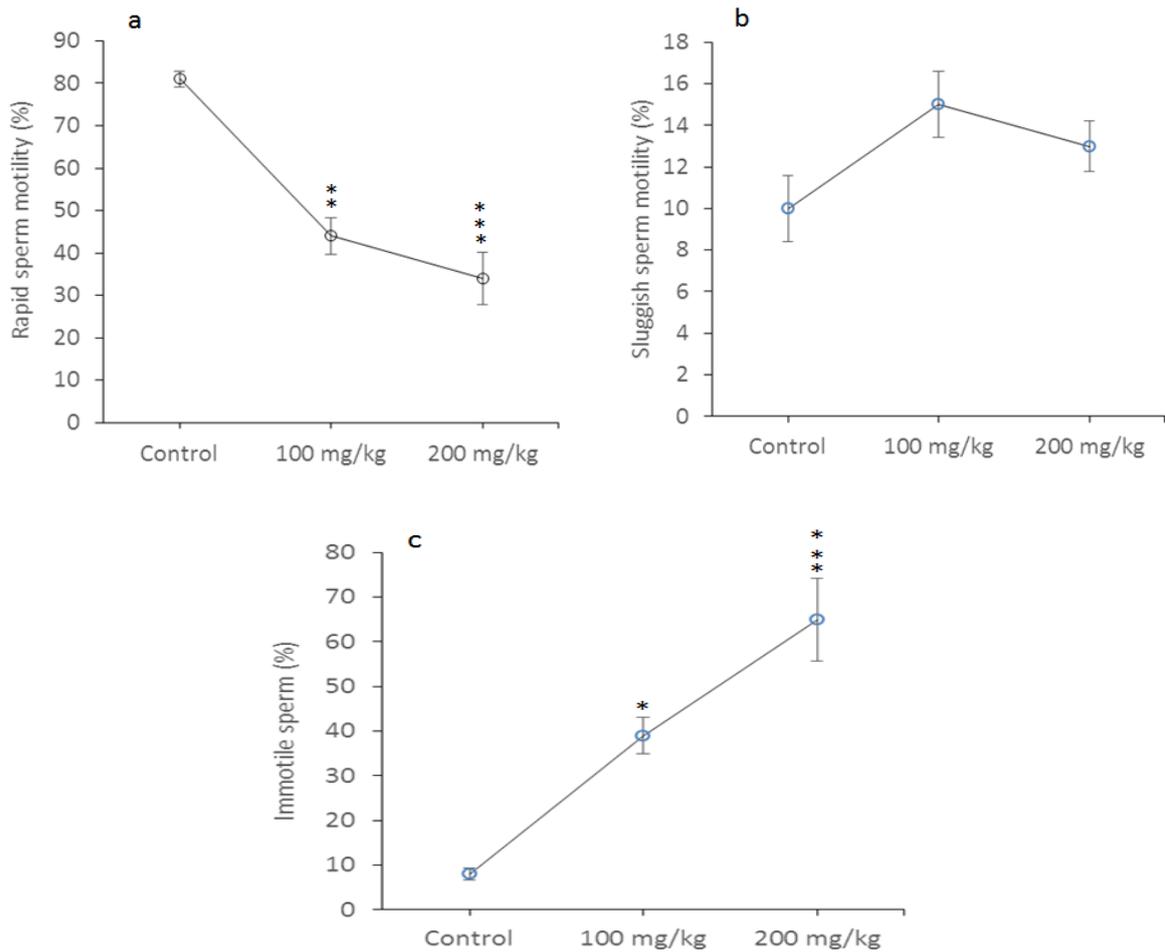
### Effects on sperm indices

Rapidly motile sperm population was significantly ( $p=0.0006$ ) decreased, whereas immotile sperm cells was increased ( $p=0.0003$ ) in extract treated rats in a dose-dependent manner when compared to control (Figures 1a and c). There was no change in percentage of sluggishly motile sperm cells among extract and control animal groups (Figure 1b). In addition, sperm count was significantly ( $p<0.0001$ ) decreased in extract administered rats in a dose-dependent manner (Figure 2a), but there was no change in sperm viability in extract treated rats compared to control (Figure 2b), Also, percentage of sperms with abnormal morphology was higher ( $p<0.0001$ ), while sperms with normal morphology was reduced ( $p<0.0001$ ) in extract treated rats when compared with control (Figures 3a and b).

**Table 1: Phytochemical constituents of aqueous *Cola acuminata* seed extract.**

Phytochemical	Result
Alkaloids	+++
Phenols	+
Tannins	++
Flavonoids	+
Saponins	++
Glycosides	+

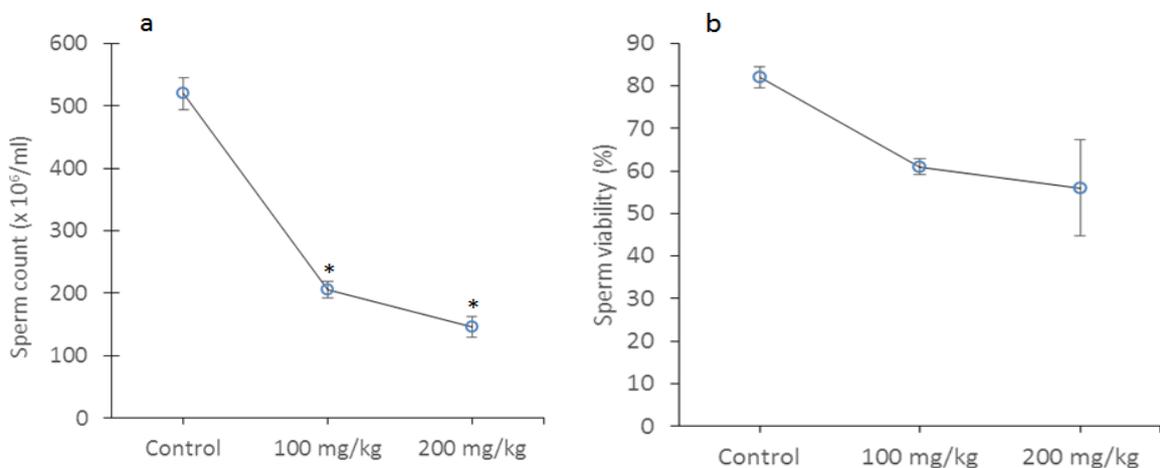
Key: + Present, ++ Moderately present, +++ Abundantly present.



**Figure 1: Effect of 30 days treatment with aqueous *Cola acuminata* seed extract on sperm motility in Wistar albino rats.**

Data are expressed as mean±Standard Error of Mean (SEM), n=5.

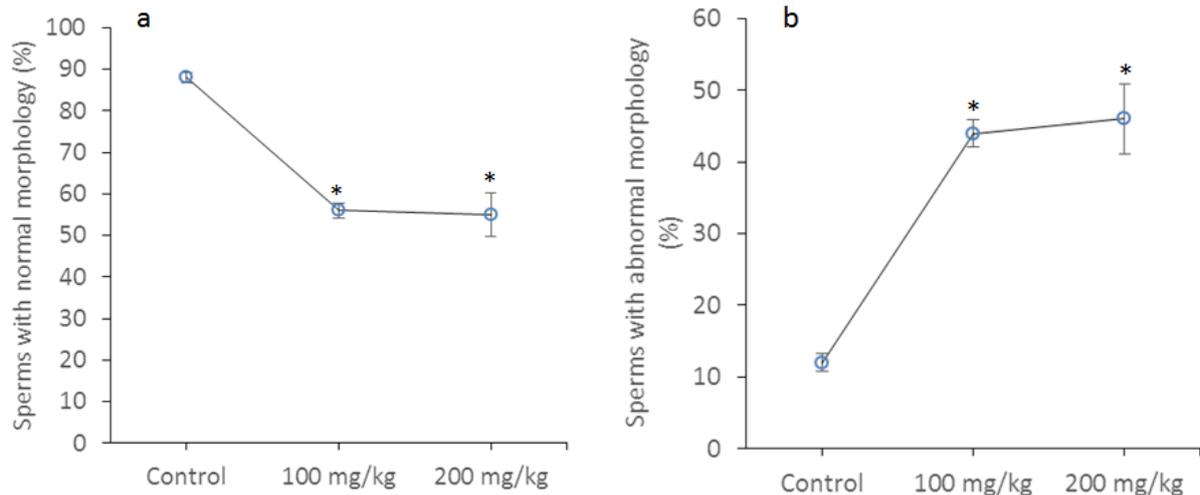
\* Significant at p<0.01; \*\* Significant at p<0.001; \*\*\* Significant at p<0.0001.



**Figure 2: Effect of 30 days treatment with aqueous *Cola acuminata* seed extract on a- sperm count and b- sperm viability in Wistar albino rats.**

Data are expressed as mean±Standard Error of Mean (SEM), n=5.

\* Significant at p<0.0001



**Figure 3: Effect of 30 days treatment with aqueous *Cola acuminata* seed extract on a- normal sperm morphology and b- abnormal sperm morphology in Wistar albino rats.**

Data are expressed as mean±Standard Error of Mean (SEM), n=5.

\* Significant at  $p < 0.0001$ .

## DISCUSSION

The effect of oral sub-acute administration of aqueous extract of *Cola acuminata* seed on sperm indices (sperm motility, count, viability and morphology) in rats was investigated in this study. The results revealed that *Cola acuminata* extract altered values of all sperm parameters that were evaluated except sperm viability. Sperm count and motility were decreased while morphological abnormality of sperm was increased. The effect was most pronounced on sperm motility. Extract administration did not affect slow motile sperms but caused a drastic reduction of rapid motile sperms and rendered most immotile. Sperm production (spermatogenesis) occurs within the seminiferous tubules in the testis,<sup>[19]</sup> and measurement of sperm count is used to assess output of the spermatogenic activity.<sup>[20]</sup> Reduction of sperm count by extract therefore indicates that it may affect the process of sperm production. Additionally, the strong inhibitory effect of extract on sperm motility suggests that the plant may interfere with microenvironment of the seminiferous epithelium and affect essential metabolic processes that involve sperm motility like calcium utilization. Further, the phytochemical analysis revealed that the extract contains high levels of alkaloids, saponins, tannins and steroids. The results obtained may thus be partly attributed to the high content of alkaloids and saponins which are believed to adversely affect testis and spermatozoa function.<sup>[21,22]</sup>

Sperm parameters are important physiological indices of sperm quality.<sup>[20,23]</sup> Although, more specific indices like fructose content, acrosome reaction, DNA fragmentation, etc are necessary to make a strong prediction of sperm quality,<sup>[24]</sup> alteration of sperm parameters by extract in the present study provides preliminary evidence of the plant's potential to affect sperm quality. Future studies are necessary to explore more markers or indices to provide understanding of mechanism of toxicity of the

plant. Added to this, there may be need to evaluate effects of longer duration of exposure, and lower dose levels of the plant.

## CONCLUSION

This study shows that sub-acute administration of *Cola acuminata* seed extract does not affect sperm viability, but decreases sperm count, motility and normal morphology in male rats.

## ACKNOWLEDGEMENTS

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## CONFLICTS OF INTEREST

None.

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